Distribution of Aflatoxin in Pistachios. 3. Distribution in Pistachio Process Streams

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The distribution of aflatoxin concentration has been measured by sampling 19 process streams ranging from 1 to 1000 nuts/sample. The 19 streams comprise substantially the entire sorted product of one producer for crop year 1992. Similar results were obtained by another producer for 1993. The fraction of total aflatoxin accounted for by each process stream was computed. It was concluded that 90% of aflatoxin is contained in 4.6% of (low-quality) product; removal of this product would reduce average aflatoxin from 1.2 to 0.12 ng/g for all product sold for human consumption. On the basis of the aflatoxin distributions of the processed as well as an unprocessed stream, the conclusion is drawn that all aflatoxin found here arises in the orchard; none is produced under normal processing conditions. It is estimated that the necessary sample size (in terms of the sample mean and desired variance of the mean) for aflatoxin measurement in pistachios is given by number of nuts = (8×10^5 ng/g) × mean/variance.

Keywords: *Processing; tree nuts; removal; process control; sampling; growth model; aflatoxin analysis*

INTRODUCTION

Tree nuts, and in particular pistachios, are subject to infection by molds, two of which, *Aspergillus flavus* and *Aspergillus parasiticus*, may give rise to the deleterious mycotoxin, aflatoxin. Because of the carcinogenic nature of aflatoxin, national organizations as well as buyers commonly limit the acceptable level of this toxin; thus, the guideline level set in the United States by the Food and Drug Administration (FDA) for human consumption is 20 ng/g (nut plus shell basis), while European consuming countries commonly set levels at 1 ng/g (B_1) and 4−5 ng/g (total). For export, as well as human health reasons, it is desirable to have the level in a lot as low as possible. This paper discusses sorting techniques that allow aflatoxin reduction to export levels.

Schatzki (1995b) noted that the aflatoxin level in raw ("as received at the processing plant") pistachios was several times higher than that in finished pistachios. Finishing pistachios involves removing a number of process substreams of low-quality product from the main process stream obtained from storage. It follows that at least some of the low-quality substreams must contain the bulk of the aflatoxin arriving from the field. Most of this low-quality product is currently sold for human consumption (albeit at a lower price). It is desirable to measure the aflatoxin content in each substream with the possible goal of eliminating most of the aflatoxin in food by discarding selected substreams.

Although there are differences in detail in the way different processors sort the product, most processors follow a scheme similar to the one shown in Figure 1, which refers to the process used by a specific processor, here referred to as processor A. Pistachios, after removal from the trees by shaking, are brought to the plant, separated from debris and superficially cleaned,

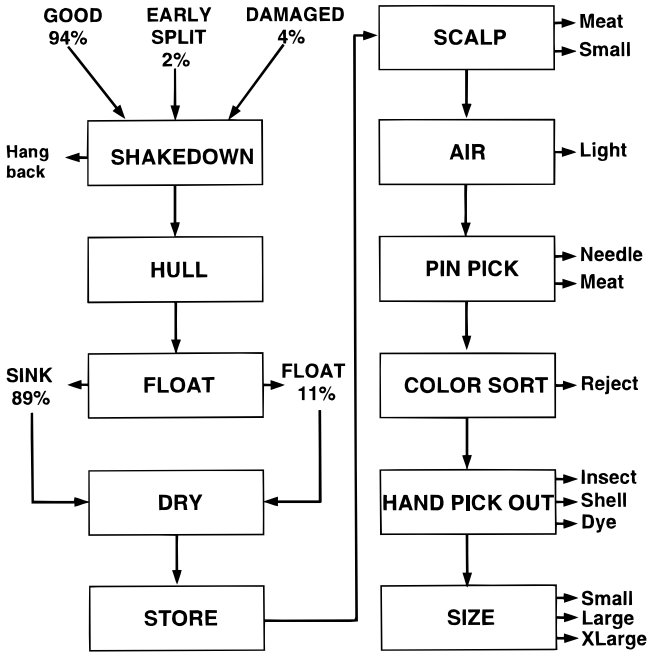

Figure 1. Process Flow.

mechanically wet hulled, and partitioned by water flotation. Shakedown at harvest tears the hulls, allowing the torn hull to ooze tannin onto the white shells and staining them or causing the hull to adhere to the shell. A similar situation arises if the hull splits before harvest (Sommer et al., 1986). [See Schatzki (1995b) for a discussion of early split versus damaged nuts.] Since white, smooth shells are desirable for product quality, effort is made to complete the hulling as quickly as possible after harvest; 6 h from shakedown to hulling is typical. Forced hot air drying follows immediately to reduce the nut water content to a level allowing long-term storage without mold production, followed by storage. Floaters and sinkers are kept separate following flotation.

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Final sorting occurs throughout the year. A scalper removes very small (>35 nuts/oz) nuts and most of the free meat as well as any remaining debris. Air flotation removes light material, mostly empty shells and shell fragments. Next a rotating drum with interior needles removes all nuts with partially open shells, which is the desirable form for a high-quality product. Any remaining free meat and nuts with unopened shells remain behind. These nuts are shipped overseas to be manually cracked by low-cost labor and reimported. In the next stage the nuts with opened shells are sent through an electric eye sorter to remove those with yellowed or stained shells, which are either shelled to produce lower value meats or sent to dyeing stage to produce a red pistachio, again of lower value. Since electric eye sorters are not foolproof, the accepted product is passed over manual sorting [hand pick out (HPO)] tables to further remove stained nuts or ones with adhering shell material. Any nuts showing obvious insect infestation are sorted out and discarded. Finally, size sorting is carried out by screening, although size is characterized by weight.

Processor A, from whom the data for Figure 1 were obtained, processes floaters and sinkers in the same way, obtaining thus 20 substreams, 10 each from floaters and sinkers. Other processors may shorten the sort for floaters, which are inherently of lower quality. There is also at least one processor who does not use flotation.

MATERIALS AND METHODS

Sample Source. Processor A receives pistachios from a large number of growers, located throughout California. Product reaches the processor generally within 24 h or less after harvest. Processors do not keep nuts from different growers separate after drying. Seventeen sublots, weighing 4.5 or 22.5 kg each, were drawn by processor A from the actual sorted substreams of 1992 crop nuts. Each sublot corresponded to a single process substream, except that no HPO insect floaters were available and meats and in-shell nuts from the scalper had not been separated. This latter separation was carried out manually, yielding 19 sublots for study. Samples of these sublots are depicted in Figure 2. To maximize information about lot distributions, particularly at the lower aflatoxin levels (Schatzki, 1995a), it was decided to analyze $N = 20$ samples from each sublot at $n = 10$ nuts each, 20 samples at $n = 100$ nuts each, and as many samples as possible of 1000 nuts each. In addition, some samples at $n =$ 1 and 10 000 were run as well.

Additional sublots were obtained from another processor, B, of 1993 crop. Processor B draws most of its raw material locally, initiating processing generally within 6 h of shakedown. Finally, analyses on processor B 1993 process streams were carried out by processor B directly, using the methods described here. Details of these sublots and results are discussed below.

Aflatoxin Determination. A method for aflatoxin analysis was required that was capable of handling samples of 1−10 000 nuts, with a dynamic range of $0.1-10^6$ ng/g and a precision of at least 30% of the measurement throughout. The large sample load made speed of analysis and minimization of waste solvent disposal essential. The protocol developed was a modification of the USDA/FGIS protocol for aflatoxin determination in grain (USDA, 1992) and is given in detail in the Supporting Information and on the anonymous ftp server aggie.pw.usda.gov as file /pub/dropbox/wrrcprot.txt and is shown as a flow diagram in Figure 3.

This modification required calibration. The entire sample was ground using a kitchen-type blender and sift mixed. A solid aliquot corresponding to the weight of 10 nuts (or total sample if less) was withdrawn and extracted. To maximize sensitivity, 90% of the entire liquid extract was used. An affinity column (fresh column for each experiment) (VICAM L. P., Somerville, MA) allowed rapid stripping of the up to 70 cm^3 or so of water/methanol extraction mixture, requiring simply the evaporation of 2 cm^3 of acetonitrile instead. Sample cleanup was a bonus. This technique, however, required passing up to 140 cm^3 of water and methanol through the column, approximately 10 times that contemplated in the column design and 50 times that used in the USDA/FGIS procedure. This resulted in a considerably lower recovery of aflatoxin, which was corrected for by calibrating the affinity column as follows. Clean (aflatoxin-free) nuts were extracted in the standard way. The recovered extraction fluid was spiked with crystalline aflatoxin (Sigma Chemical Co., St. Louis, MO). B_1 and G_1 were dissolved separately in MeOH/aqueous at 0,0; 0,120; 120,0; 60,60; and 120,120 ng and total volume of 5, 15, 25, 35, 50, and 60 cm^3 (before dilution with water) and passed through the remaining steps of the protocol (affinity column, derivatization, HPLC). The resulting HPLC areas were fitted separately for B_1 and G_1 recovery by SAS GLM procedure *(SAS/STAT User's Guide,* 1988). No attempt was made to calibrate the affinity column for B_2 or G_2 as peaks corresponding to these compounds were virtually never seen. This calibration was repeated without the use of affinity columns to evaluate their contribution to the analytic error.

To achieve the dynamic range desired from a single solid aliquot of the ground sample, the following procedure was used. After precolumn derivatization, the sample's fluorescence was visually compared (using safe light, see Supporting Information) to a daily prepared standard (100 ng total). If column saturation was indicated, a set of 10-fold dilutions of the extract was run instead. Since the HPLC analysis had a dynamic range of 130−220-fold (see below), at least two diluted samples were always in range for comparison and the desired dynamic range of 7 decades was obtained. Analysis was conveniently done by a PC spread sheet program (Lotus 123, Lotus Development Corp., Cambridge, MA). The amount of aflatoxin reported was based on the volume of extraction solvent used, rather than the concentration in the recovered solvent, so that the amount of extracted toxin remaining behind in the cake is accounted for. This differs somewhat from what is commonly reported. The recovered volume amounted to 74 ± 6% of the extraction volume. Concentration is expressed as nanograms of aflatoxin (G_1 plus B_1) per gram of nut (meat plus shell).

RESULTS

Calibration, Error Analysis. Calibration of the complete analytical system (affinity column through HPLC) was carried out by spiking with B_1 and G_1 as described above. For a given *volume* passed through the column, HPLC *area* and reproducibility were found to be proportional to the amount (*ng*) of spiked aflatoxin. Increasing volume decreased recovery, with a plateau being approached at the upper limit of volume used. Accordingly, the B_1 data were fit to

$$\log(area_B) = a_{B0} + a_{Bn}\log(ng_B) + a_{Bv}volume + a_{Bvv}volume^2 + a_{BG}ng_G$$

where the a_{BG} term accounts for possible absorption site competition between the aflatoxins. A similar expression for the HPLC *area* corresponding to aflatoxin G_1 was used. Analysis was done using the SAS GLM procedure. Only the first four terms were significant at the $P = 0.05$ level; no significant site competition was noted. As expected, a_{Bn} and a_{Gn} were found to be close to unity (0.974 and 1.003). Root mean square (rms) errors of 0.11 for $\log(area_B)$ and 0.16 for $\log(area_G)$ were obtained. These coefficients of variation (11 and 16%) represent the analytic error of the method.

To test whether the above error arose from the affinity column or other parts of the procedure (HPLC, dilution,

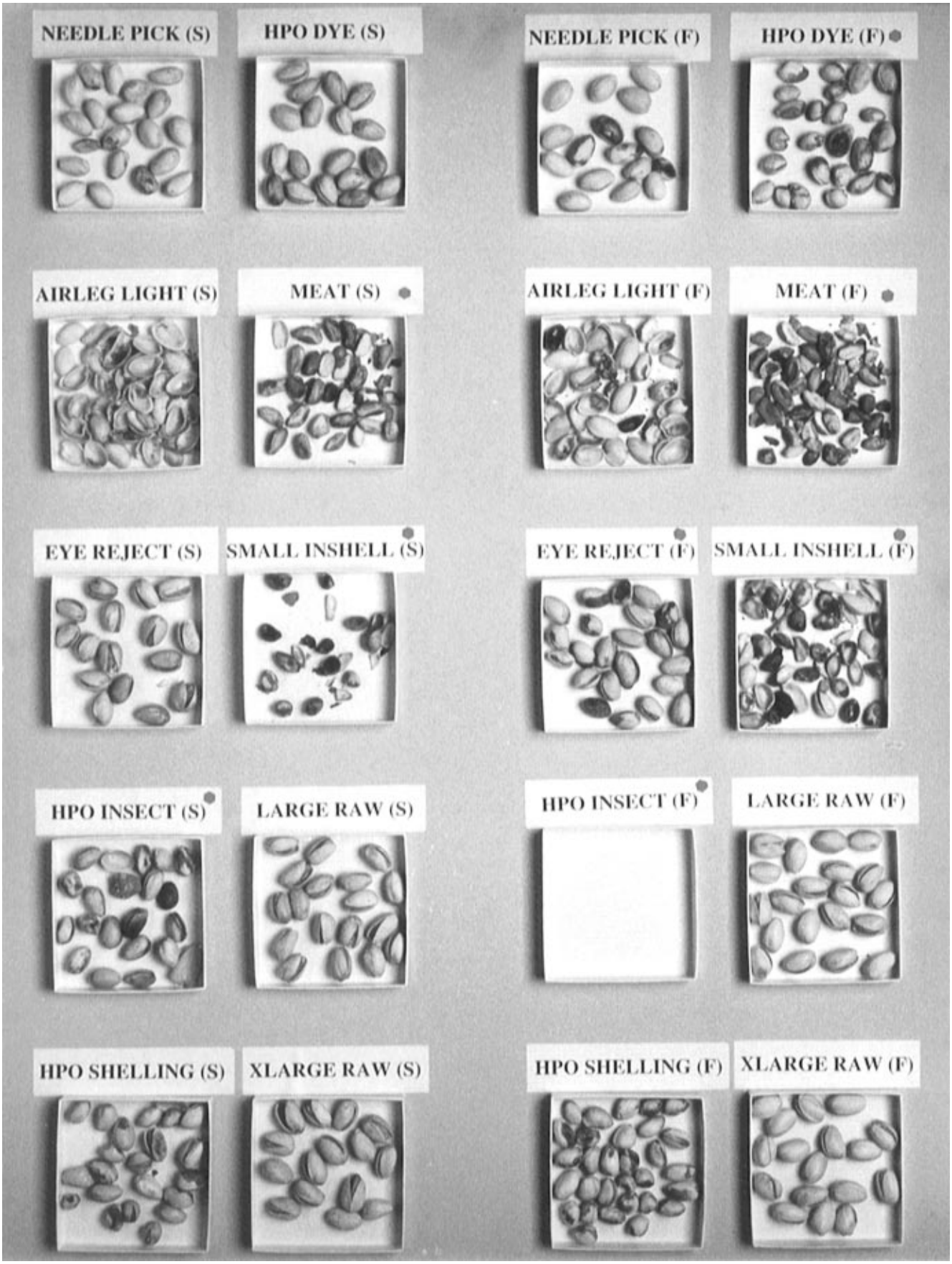

Figure 2. Samples from the 19 analyzed process streams.

derivatization), this calibration was repeated using aflatoxin spikings ranging from 1 to 120 ng each, but without the use of the affinity column and without the use of the volume terms in the fitting equation.

In this case rms errors of 0.03 and 0.05 were obtained for $\log(area_B)$ and $\log(area_G)$, respectively. Thus, substantially all of the analytic error is assigned to the use of the affinity column. In testing the HPLC column it

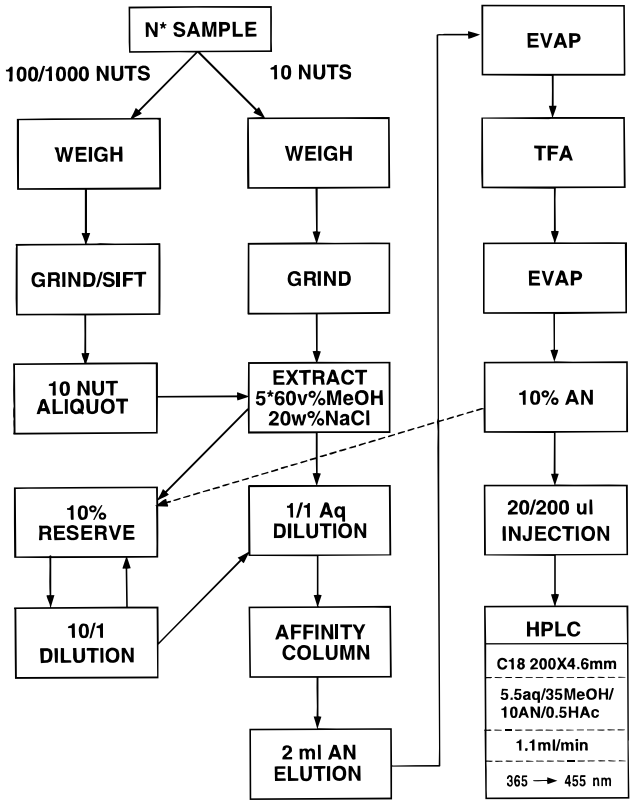

Figure 3. Analysis protocol.

was noted that aflatoxin would adsorb on newly purchased glassware (Haddon et al., 1977). This problem was solved by silination (surface deactivation) of the volumetric flasks using Sigmacote (Sigma Chemical Co., St. Louis, MO; instructions available from Sigma). The lower limit of detectibility amounted to an area corresponding to about 0.02 ppb.

Duplicated liquid aliquots, derived from the same extract but at various dilutions, showed a pooled coefficient of variation (PCV) of 17%, which includes the analytic error. The increase from total system calibration might be caused by the multiple dilution. Duplicated solid aliquots after sift mixing (100 or 1000 nuts) had a PCV of 11%, which represents subsampling as well as analytic error. Duplicate samples taken after tumble mixing (10 000 nuts) showed a PCV of only 2%. One concludes that the subsampling error is somewhat smaller than the analytic error.

Sample Results, Lot Distributions. The sample results are shown in Tables 1−4, binned logarithmically into semidecimal bins, following the method of Schatzki (1995a). On the basis of a 17% precision, a bin size corresponding to $\log_{10}2$ is justified. However, the number of samples run, N, for each process stream was too small to obtain an adequate estimate of the probability $P_i(n)$ corresponding to each bin i. In the case of the meat samples, the obtained values were halved before binning to take into account the missing shells, which generally weigh about the same as the meat and contain little aflatoxin. Division of each row of Tables 1−4 by the total number of samples in the row yields the estimated sample probability distribution $\{\hat{P}_i(n)\}$. (The expression {...} stands for "the set of all ...".) In the case of the eye reject floaters a part of the sublot had been sorted into two parts and each part was analyzed separately in 1-nut samples for other work. The resulting distributions were recombined at the p_i level (see next paragraph).

Sample probability distributions may be converted to lot probability distributions, $\{p_i, c_i\}$, following the method shown in Schatzki (1995a). Here p_i is the probability that a single nut in the lot (not the sample) has aflatoxin concentration c_i. Logarithmic p_i vs c_i plots for processes showing appreciable contamination, derived from Tables 1−4, are shown in Figure 4. The $\{\hat{P}_i(n)\}$ distributions for meat floaters did not meet the assumptions made in Schatzki (1995a). A narrow peak was noted centered at the bin $0.1 < C < 0.32$ ng/g, independent of n, along with substantially no uncontaminated samples. This situation indicated that in this case p_i exceeded 0.1 for $c_i = 0.18$. Hence, samples for which $n > 10$ and $C < 0.32$ ng/g should have a representative number of infected nuts. This is shown symbolically by the vertical arrow in Figure 4a. At high C, $n < 1/p_i$; hence, the distributed Poisson distribution should apply (Schatzki, 1995a), yielding the usual broad distribution.

The average aflatoxin concentration for each process stream and its standard error is given in Table 5, in each case computed from the measured sample concentrations for the highest available $N \times n$ to minimize the variance. In the same table this result is combined with the size of the total process stream, as supplied by processor A, to yield the fraction of the total aflatoxin accounted for by each process stream.

Processor B. To ascertain whether the above results could be generalized, corresponding data were obtained from another processor in California in another crop year, 1993. Processor B followed substantially the process shown in Figure 1, except that a much lighter cut was taken with the electric eye sorter, removing but 1.4% of the sinker stream rather than 12%. Slightly different cuts on the basis of nut size were taken as well, but these could be characterized in terms of the weight/nut. Processor B measured the aflatoxin content of each of the sinker streams obtained, after storage. Up to 20 samples of 1500 g (approximately 1000 nuts) each were taken from each stream and analyzed following the protocol given in the Supporting Information. However, grinding and mixing were inadequate, as was verified by inspection. Inadequate grinding and mixing result in subsampling errors and the sample size n cannot be defined. The resulting data cannot be used for distribution calculations, which require a constant n. On the other hand, the sample average is independent of n (although the variance will not be), so that the sample average $\langle C \rangle$ estimates the lot average $\langle c \rangle$. Accordingly, processor B's $\langle C \rangle$ results are listed in Table 5 as well. Where necessary, sample streams have been combined to match processor A's description. Aflatoxin measurements for floaters were not available for processor B. To obtain an estimate of the fraction of total aflatoxin present in each product stream, the last column of Table 5c was computed on the assumption that the flotation process partitioned aflatoxin as it did for processor A.

Two sublots, corresponding to the large and extra large sinkers of Table 5, were obtained from processor B and their aflatoxin levels measured to obtain a comparison between the two laboratories. The average level of aflatoxin measured for ten $n = 10\,000$ samples was 0.63 ± 0.90 ng/g for large sinkers and 0.04 ± 0.13 ng/g for extra large sinkers, in agreement with the values obtained by processor B (0.59 and 0.22 ng/g, respectively).

Table 1. Aflatoxin B_1+G_1, Processor A, 1992: Number of 1-Nut Samples in Each Range

ng/g[a]	none	<0.1	<0.32	<1.0	<3.2	<10	<32	<100	<317	<1000	<3170
sinkers											
X large	100										
floaters											
eye rej, suture stain (12%)	42		1	15	5						
eye rej, nonsuture stain (88%)	33		5	3			1				

[a] In Tables 1−5 the symbol < in the heading indicates a range. Thus, <0.1 indicates 0.03−0.1, <0.32 is shorthand for 0.11−0.32, <1.0 for 0.33−1.0, etc.

Table 2. Aflatoxin B_1+G_1, Processor A, 1992: Number of 10-Nut Samples in Each Range

ng/g	none	<0.1	<0.32	<1.0	<3.2	<10	<32	<100	<317	<1000	<3170
sinkers											
meats	20										
scalpers	16		2	2							
needle rej	20										
air light	20										
eye rej	20										
HPO insect	16		3	1							
HPO shell	20										
HPO dye	20										
large	20										
X large	97		3								
floaters											
meats	3	1	14	2							
scalpers	18		2								
needle rej	20										
air light	11		3	4	2						
eye rej	13	1	4	2							
HPO shell	18		1	1							
HPO dye	13	1	5		1						
large	17	3									
X large	17	2	1								

Table 3. Aflatoxin B_1+G_1, Processor A, 1992: Number of 100-Nut Samples in Each Range

ng/g	none	<0.1	<0.32	<1.0	<3.2	<10	<32	<100	<317	<1000	<3170
sinkers											
meats	17	1	2								
scalpers	19							1			
needle rej	20										
air light	19		1								
eye rej	20										
HPO insect	17		1			1					1
HPO shell	20										
HPO dye	20										
large	17	2	1								
X large	20										
floaters											
meats		1	17	1	1						
scalpers	6		4	6					1	1	2
needle rej	20										
air light	15		2	3							
eye rej	14	1	2	1							2
HPO shell	19	1									
HPO dye	9		6	1		1	2				1
large	17	1	2								
X large	18	2									

Table 4. Aflatoxin B_1+G_1, Processor A, 1992: Number of 1000-Nut Samples in Each Range

ng/g	none	<0.1	<0.32	<1.0	<3.2	<10	<32	<100	<317	<1000	<3170
sinkers											
meats	9	5	3	1			1	1			
scalpers	1			2		1	1			1	
eye rej	12						1				
large	14										
X large	11										
floaters											
meats	1	1	4	5	1		1	3	1		
scalpers	4		1	2	1	2		3		2	1
eye rej	11			1				2			
large	7	3	1						1		
X large	13						1				

Aflatoxin B_1/G_1 Relations. Dorner et al. (1984) report that *A. flavus* generally produces only aflatoxins B_1 and B_2, while *A. parasiticus* produces both aflatoxins B and G. Although we report only B_1+G_1 in detail, the relative amount of the two aflatoxins is thus of some interest. Of the samples reported in Tables 1−5 80% were negative, two or 0.2% showed only G_1 (0.20 and 0.26 ng/g), 9% only B_1, and 11% both B_1 and G_1. Four

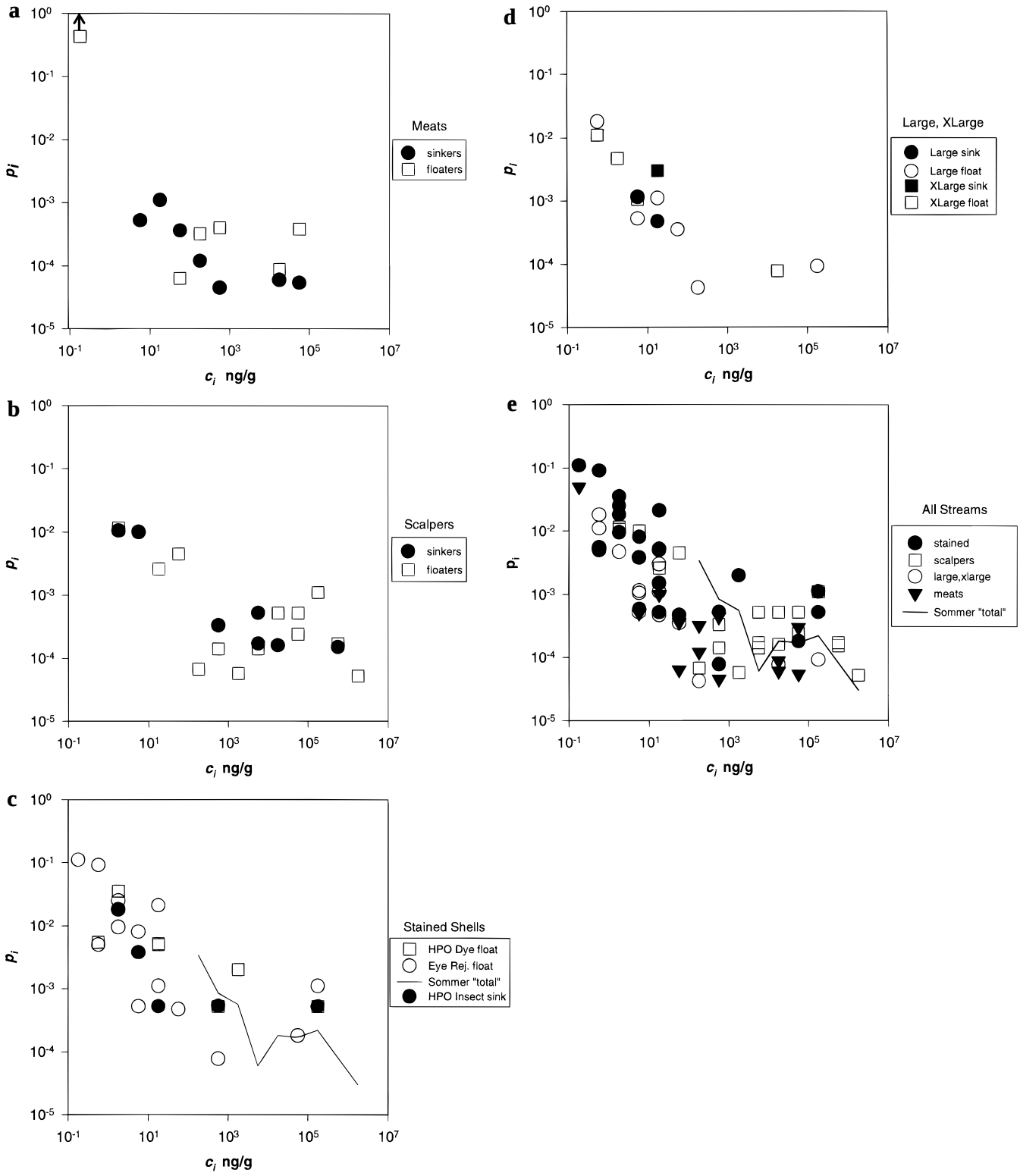

Figure 4. Single nut probability distributions for selected process streams: (a) meats; (b) scalpers; (c) stained shell nuts; (d) large and extra large nuts; (e) all infected process streams.

percent of the samples had B_1 and $G_1 > 0.3$ ng/g; for these B_1/G_1 ranged from 0.2 (90/390) to 41 (135/3.3) with an average of 3.8.

DISCUSSION

Lot Distributions. The lot probabilities p_i,c_i are derived from the estimated sample probabilities to a first approximation as $p_i = \hat{P}_i(n)/n = N_i(n)/N(n)/n$ and $c_i = C_i n$. Here $N_i(n)$ is the number of samples [of $N(n)$] that fall into bin i, and C_i is the midpoint of bin i (Schatzki, 1995a). Thus, one may obtain $\{p_i,c_i\}$ values only if they fall into a band given by $-\log n - \log N(n) < \log p_i < -\log n$ and $\log c_i \geq \log C_0 + \log n$, where C_0 is the lowest detectable aflatoxin concentration. It is for this reason that one needs to run experiments at a set of n values to cover a large range of $\{p_i,c_i\}$. In the present case, where $N(n) \leq 20$ and n occurs in steps of 10, these bands barely overlap and each p_i is derived

Table 5. Aflatoxin Content of Process Streams

product	⟨C⟩, ng/g	% product	% aflatoxin
(a) Processor A, 1992: Sinkers			
meats	4.9 ± 17	1.77	7.0
scalpers 40/50	91 ± 207	0.53	39.5
needle	0	10.38	0
air light	0.02 ± 0.07	2.04	0.0
eye rej	1.0 ± 3.7	10.91	9.1
HPO insects	92 ± 408	0.89	67.2[a]
HPO shell	0	0.89	0
HPO dye	0	3.02	0
large 21/22	0	31.06	0
X large 18/20	0	28.39	0
sink total	0.76	89	55.6
(b) Processor A, 1992: Floaters			
meats	38 ± 80	0.12	3.8
scalpers 60/70	149 ± 316	0.10	12.2
needle	0	7.63	0
air light	0.1 ± 0.2	0.13	0.0
eye rej	7.8 ± 18	1.55	9.9
HPO shell	0.001 ± 0.004	0.16	0.0
HPO dye	135 ± 593	0.13	14.4
large 18/20	9.6 ± 33	0.44	3.5
X large 18/20	0.9 ± 3.4	0.73	0.5
float total	4.9	11	44.3
grand total	1.22	100	100
(c) Processor B, 1993			
sinkers			
scalpers, incl meats >30	24.11	1.31	21.4
needle	0.15	12.55	1.3
eye rej (dye)	4.82	1.15	3.8
HPO dye	17.32	0.35	4.1
HPO shell 30[c]	23.72	0.15	2.4
HPO shell[d]	0.74	0.19	0.1
small 26/30	3.11	1.18	2.5
large 23/25	0.59	30.94	12.4
X large 18/22	0.22	37.17	5.4
sink total	0.93	85	53.4[b]
float total	4.58[b]	15	46.6[b]
grand total	1.48[b]	100	100

[a] Not in total. [b] Based on the assumption that flotation partitioned aflatoxin as for processor A. [c] Picked from 21/25 eye accept stream. [d] Picked from 18/20 eye accept stream.

from 20 or fewer samples. As a result, the scatter in {log p_i, log c_i} plots is quite large. In addition, $\hat{P}_i(n)/n = p_i = 0$ points are not represented; adjacent points will have a positive bias (see e.g. the large floater data in Table 4).

Aflatoxin Measurement. Given the aflatoxin distribution, a rough estimate can be made of the sample size needed to yield a desired precision in estimating aflatoxin lot content. From the expression for mean and variance of C (which are equal to $\langle c\rangle$ and Var(c) and are given above) one obtains $nN = \langle C\rangle/\mathrm{Var}(C)\ \sum_i p_i c_i^2/\sum_i p_i c_i$ for the number of nuts needed. The sums are strongly weighted toward large i, since c_i increases rapidly with i, while p_i is roughly constant at large i. The ratio of the two sums can be estimated as $0.8c_i(max)$, where $c_i(max)$ is the largest c_i before the probabilities drop to zero. From the discussion given below and Figure 4e, $c_i(max) \approx 10^6$ ng/g within a factor of 2 or so. Hence, one obtains for the number of nuts needed

$$nN = 800000\langle C\rangle/\mathrm{Var}(C) \qquad (1)$$

For a 20 ng/g level and a standard error of 10 ng/g, nN becomes 160 000 nuts or about 500 lb (220 kg) (21 nuts/oz basis)!

The validity of expression 1 can be tested if $\langle C\rangle$ and Var(C) are available experimentally. Four pistachio sample sets were discussed in Schatzki (1995b). Computing $nN \times \mathrm{Var}(C)/\langle C\rangle$ for these sets, one obtains 8.4 $\times 10^5$, 2.6 $\times 10^6$, 1.6 $\times 10^5$, and 2.9 $\times 10^5$ ng/g for the Sommer "total", DFA 1983−1986, DFA 1990−1991 (raw), and DFA 1990−1991 (finished) populations, respectively. These values are in fair agreement with the estimated value of 8 $\times 10^5$ ng/g, particularly for the Sommer data. Whitaker et al. (1994) studied a set of 40 lots of peanuts, repeatedly sampling at n = 6097, 3724, and 1999 pods. They fitted all $\langle C\rangle$ and Var(C) data to $\mathrm{Var}(C) = (95.4 \pm 0.5) \times \langle C\rangle^{0.96\pm0.08}$ for n = 6097. The predicted proportionality of $\langle C\rangle$ to Var(C) is almost exactly obtained. Writing $nN \times \mathrm{Var}(C)/\langle C\rangle = 6097 \times 95.4 \times \langle C\rangle^{-0.04}$ and approximating $\langle C\rangle$ in the last factor by the overall mean of $\langle C\rangle$ (800 ng/g), one obtains 4.45 $\times 10^5$ ng/g. Within the noted accuracy expression 1 can serve to set regulations or experimental designs prior to actual sampling.

Aflatoxin Production Model. Inspection of Figure 4, and in particular Figure 4e, reveals that the lot distributions {ln p_i, ln c_i} estimated here have the following form: a rapid, roughly linear drop from about c = 0.3−10^3 ng/g, followed by a plateau (possibly somewhat elevated at the center) for $c = 10^3$−10^6 ng/g and a sudden sharp drop around 10^6 ng/g. The height, particularly in the plateau region, depends on the type of stream, decreasing in the order stained, scalpers, meats, (X)large. The Sommer "total" data [Sommer, 1986, as calculated by Schatzki (1995b)], which is based on early split nuts, show a similar shape. It is of interest to relate this general shape to the physical processes believed to be responsible for aflatoxin production.

The drop-off around 10^6 ng/g appears to represent the upper limit of aflatoxin that a single kernel can produce, about 1 mg. This concentration seems to be similar for all tree and ground nuts. The highest level reported for aflatoxin in a single nut is 4 $\times 10^6$ ng/g in parts of a peanut (Cucullu et al., 1966). All reported pistachio data for n-size samples, including those reported here, show essentially no samples greater than a few times $10^6/n$ ng/g (Schatzki, 1995a,b, unpublished results; Sommer et al., 1986).

The level of the {p_i} data in the intermediate region, $10^3 < c_i < 10^6$ ng/g, was seen to be process stream dependent. Cross-contamination in processing or during storage, if any, should have similar effects on all nuts that are stored together until sorting. (Floaters and sinkers are stored separately, but under identical conditions.) One concludes that the aflatoxin contamination represented by the plateau region must occur before harvest, with an intensity presumably related to the order of the height of the plateau noted above. Staining, in particular, is associated with hull splitting in the orchard, the tannin from the hull tear causing discoloration. The plateau region is thus tentatively assigned to early hull splitting. Sommer et al. (1976) found the log of the aflatoxin concentration in inoculated pistachios to be linear with length of infection, as would be expected for a self-propagating process. The horizontal axis in Figure 4 would thus be linear in the time between hull split and harvest, the earlier split nuts having the higher final concentration. To obtain a scale

it is noted that Doster and Michailides (1993) observed that early splitting in pistachios occurred during a 6-week period prior to harvest, with a maximum rate occurring around 4 weeks. The region from 10^3 to 10^6 ng/g would then represent the time of mold infection of early split or otherwise weakened nuts (possibly caused by insect or bird attack); in the present case a 1000-fold increase in 6 weeks represents a rate 4 times that noted by Sommer et al.

The rise of p below $c = 10^3$ ng/g appears to be present in most process streams tested. It may be due to a process common to most nuts in the orchard, such as late hull splitting ("tattering"), or it might be due to cross-contamination in the hullers or flotation baths (the moisture levels during storage are too low to allow aflatoxin production). It is of interest that meats show the highest frequency at low concentration, while the closed-shell needle pick reject nuts show none. This suggests that contamination, if any, selectively occurs on meat material. Tests on a limited number of nuts that had been collected after harvest, but before any processing took place, did find such a rise at low c, but at a lower level than that shown in Figure 4. The beginning of a rise is seen in the Sommer data, which were based on nuts picked off trees. A clear decision of this question would require testing of a much larger sample of unprocessed nuts.

The average aflatoxin level in a process stream is essentially due to the p_i level in the $10^3 < c_i < 10^6$ ng/g region. Two processing vectors are seen to influence large aflatoxin levels: shell staining and floating. These conclusions are supported by the results of Table 5, noted previously. Shell staining is presumably related to hull tearing before harvest, as the torn hull oozes discoloring tannin. Indeed, the {ln p_i, ln c_i} distributions of early splits and stained nuts in Figure 4 match reasonably closely, as do the actual amounts of material, 2.57% (Table 5) and 2% (Sommer et al., 1986) of product. This suggests that stained and insect infected nuts are derived from early split nuts. The scalpers show a {ln p_i, ln c_i} distribution similar to early split and may derive from the weakened and damaged nuts. Pearson (1993) showed that early splits nuts were smaller. From Table 5 it is also clear that the floater streams invariably contain more aflatoxin than the corresponding Sinker streams. The physical basis for this is not understood at this time. The needle sorter reject nuts show no aflatoxin contamination at all as do the air light sinkers (almost). The former only present a closed shell, while the latter consist almost entirely of shell material. (Note that needle reject refers to nuts in the process stream, not nuts returned after water soaking and cracking overseas.) Aflatoxin is not produced by mold on the shell.

Process Control. The results of Table 5a,b suggest a straightforward way to reduce total aflatoxin in product destined for human consumption; one must discard selected product streams. On the basis of the results obtained for processor A, the following conclusions can be drawn. The aflatoxin is largely contained in the stained nuts, which include the scalpers, the eye rejects, the HPO insects, the HPO dye floaters, and the meat sinkers. (The HPO shelling stock consists largely of adhering hull, not stained, product.) (See Figure 2.) By discarding all scalpers and HPO dye floaters, one reduces aflatoxin levels by 66% while rejecting <0.8% of total product. Discarding eye reject floaters and large floaters as well reduces aflatoxin another 13% at a total cost of 2.75% of product, and if all scalper meats (not meats resulting from shelling) are discarded as well, aflatoxin is reduced to 10% of the original while a total of 4.64% of product is lost. Furthermore, the discarded material is precisely the low-cost product so that the loss of income is well under 4.6%.

Even the streams suggested for discarding contain only a small fraction of infected nuts. Such streams are therefore candidates for resorting, which could salvage an appreciable fraction of product without reintroducing aflatoxin into material scheduled for human consumption. Such resorting techniques are the subject of active research in our laboratory (Keagy et al., 1994, 1996; Pearson, 1994, 1996).

Processor B. It is of interest to establish whether the conclusions regarding process control are restricted to a particular processor and crop year. Since values for the floater streams for processor B are not available, we restrict our comments to sinkers only by comparing parts a and c of Table 5. Although Table 5c is based on aflatoxin measurements done by processor B, while Table 5a is based on Tables 1−4 done at our laboratory, we showed above that the results of both laboratories are comparable. Further, the two processes yielded substantially the same product breakdown with two exceptions: eye reject 10.9% vs 1.2% and HPO 3.9% vs 0.7%. Total sinkers are also very similar, 89% vs 85%. The significant difference between the two processors is the result of the substantially deeper cut taken by processor A in eye color sorting and subsequent hand sorting. The result of this is seen in the aflatoxin level of the large and extra large nuts, 0 and 0 ng/g for processor A vs 0.44 and 0.16 ng/g for processor B. Because of the large volumes involved, this final high-grade product still contained 18% of total aflatoxin for processor B, none for processor A. With this exception, it is clear that the general pattern observed for processor A carries through to another processor and another year. These conclusions are of some generality. Not only do most processors follow substantially the same process shown in Figure 1, but processors A and B jointly account for well over half the total U.S. pistachio production.

To obtain actual values for a specific process or crop year, it would still be necessary to repeat the measurements made here. In light of the required sample size, discussed above, such measurements would presumably be limited to those process streams that are suspected of having high aflatoxin content.

Supporting Information Available: Protocol used for aflatoxin analysis in pistachios (4 pages). Ordering information is given on any current masthead page.

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